REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2012	Journal Article-AmJPhysiolRegulIntegrcompPhysio	

4. TITLE AND SUBTITLE

Biological and Analytical Variation of the Human Sweating Response: Implications for Study Design and Analysis

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

R.W. Kenefick, S.N. Cheuvront, L.D. Elliott, B.R. Ely, M.N. Sawka

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Thermal and Mountain Medicine Division
U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

8. PERFORMING ORGANIZATION REPORT NUMBER

M11-43

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Same as #7 above.

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Appropriate quantification of analytical and biological variation of thermoregulatory sweating has important practical utility for research design and statistical analysis. We sought to examine contributors to variability in local forearm sweating rate (SR) and sweating onset (SO) and to evaluate the potential for using bilateral measurements. Two women and eight men (26 + 9 yr; 79 + 12 kg) completed 5 days of heat acclimation and walked (1.8 l/min V ˙ O2) on three occasions for 30 min in 40°C, 20% RH, while local SR and SO were measured. Local SR measures among days were not different (2.14 + 0.72 vs. 2.02 + 0.79 vs. 2.31 + 0.72 mg•cm2•min-1, P = 0.19) nor was SO (10.47 + 2.54 vs. 10.04 + 2.97 vs. 9.87 + 3.44 min P = 0.82). Bilateral SR (2.14 + 0.72 vs. 2.16 + 0.71 mg•cm2•min-1, P = 0.56) and SO (10.47 + 2.54 vs. 10.83 + 2.48 min, P = 0.09) were similar and differences were 1 SD of day-to-day differences for a single forearm. Analytical imprecision (CVa), within (CVi)-, and between (CVg)-subjects' coefficient of variation for local SR were 2.4%, 22.3%, and 56.4%, respectively, and were 0%, 9.6%, and 41%, respectively, for SO. We conclude: 1) technologically, sweat capsules contribute negligibly to sweat measurement variation; 2) bilateral measures of SR

15. SUBJECT TERMS

sweating rate; reproducibility; exercise-heat stress; sweating onset

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Robert W. Kenefick
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(Include area code)* 508-233-6344

Reset

Biological and analytical variation of the human sweating response: implications for study design and analysis

Robert W. Kenefick, Samuel N. Cheuvront, Leonard D. Elliott, Brett R. Ely and Michael N. Sawka

Am J Physiol Regul Integr Comp Physiol 302:R252-R258, 2012. First published 9 November 2011; doi:10.1152/ajpregu.00456.2011

Am J Physiol Regul Integr Comp Physiol 302: R252–R258, 2012.
First published November 9, 2011; doi:10.1152/ajpregu.00456.2011.

Biological and analytical variation of the human sweating response: implications for study design and analysis

Robert W. Kenefick, Samuel N. Cheuvront, Leonard D. Elliott, Brett R. Ely, and Michael N. Sawka

US Army Research Institute of Environmental Medicine, Natick, Massachusetts

Submitted 11 August 2011; accepted in final form 7 November 2011

Kenefick RW, Cheuvront SN, Elliott LD, Ely BR, Sawka MN. Biological and analytical variation of the human sweating response: implications for study design and analysis. *Am J Physiol Regul Integr Comp Physiol* 302: R252–R258, 2012. First published November 9, 2011; doi:10.1152/ajpregu.00456.2011.—Appropriate quantification of analytical and biological variation of thermoregulatory sweating has important practical utility for research design and statistical analysis. We sought to examine contributors to variability in local forearm sweating rate (SR) and sweating onset (SO) and to evaluate the potential for using bilateral measurements. Two women and eight men (26 ± 9 yr; 79 ± 12 kg) completed 5 days of heat acclimation and walked (1.8 l/min $\dot{V}O_2$) on three occasions for 30 min in 40°C, 20% RH, while local SR and SO were measured. Local SR measures among days were not different (2.14 ± 0.72 vs. 2.02 ± 0.79 vs. 2.31 ± 0.72 mg·cm^{2}·min^{-1}, $P = 0.19$) nor was SO (10.47 ± 2.54 vs. 10.04 ± 2.97 vs. 9.87 ± 3.44 min $P = 0.82$). Bilateral SR (2.14 ± 0.72 vs. 2.16 ± 0.71 mg·cm^{2}·min^{-1}, $P = 0.56$) and SO (10.47 ± 2.54 vs. 10.83 ± 2.48 min, $P = 0.09$) were similar and differences were ≤ 1 SD of day-to-day differences for a single forearm. Analytical imprecision (CV_a), within (CV_i)-, and between (CV_g)-subjects' coefficient of variation for local SR were 2.4%, 22.3%, and 56.4%, respectively, and were 0%, 9.6%, and 41%, respectively, for SO. We conclude: *1*) technologically, sweat capsules contribute negligibly to sweat measurement variation; *2*) bilateral measures of SR and SO appear interchangeable; *3*) when studying potential factors affecting sweating, changes in SO afford a more favorable signal-to-noise ratio vs. changes in SR. These findings provide a quantitative basis for study design and optimization of power/sample size analysis in the evaluation of thermoregulatory sweating.

sweating rate; reproducibility; exercise-heat stress; sweating onset

A FUNDAMENTAL CHALLENGE IN the study of human integrative physiology is in the identification and quantification of the many sources of human variability. Differences in the interpretation of an experimental effect (signal) depend largely on the measurement variability (signal-to-noise ratio), particularly where purely statistical conclusions are drawn. This is especially true in the study of thermoregulatory sweating where modifying factors, such as circadian rhythm (36, 37), sex hormones (19), hydration status (23), sleep (32), acclimation (1, 36), exercise intensity (23), and fitness status (16) must be taken into account. These factors represent preanalytical influences that researchers must control to properly reveal the functional role that other variables (e.g., drugs, age, exercise, etc.) have on thermoregulatory sweating (3). However, less appreciated are the random day-to-day fluctuations in sweating that exist within (CV_i), and between (CV_g) humans owing to analytical (CV_a) and inherent biological measurement variation. Just as the systematic study and control over preanalytical factors has helped shape, our understanding of thermoregulatory sweating, quantitative assessment of analytical and biological variation in sweating may also have important practical utility. Indeed, appropriate calculation of statistical power and/or sample size requires accurate information about variability from these three sources.

Modern ventilated sweat capsules commonly used in experimental research (8, 22, 28, 34, 39) can be placed at multiple skin sites (e.g., arm, leg, chest, back) and can be used in conjunction with other physiologically relevant measures. However, the contribution of modern ventilated sweat capsules to biological variation in sweating has never been determined despite their common use. Knowledge of CV_a is important, particularly in experimental research, as it represents the bias introduced by the instrument itself each time a measurement is made (9). Yet the accuracy and precision of sweat capsules are unreported and the measurement reproducibility of a fixed, calibrated sweating rate (SR) could be affected by hysteresis or other factors associated with sweat capsule technologies (13). Additionally, determination of the CV_i and CV_g allows quantification of the random biological noise in sweating, exhibited day to day, even after controlling for preanalytical factors. According to Brengelmann et al. (3), the reproducibility of any thermoregulatory effector response is a key to understanding the potential effects of other variables on that parameter. Biological variation not only quantifies measurement reproducibility, it also affords a probabilistic interpretation of the effect magnitude of any measured change in sweating (9). The potential utility of using biological variation to understand thermoregulatory sweating may have important practical utility for research study design (15), statistical analysis, sample size estimates, and the likelihood of type I and type II error commissions (21).

In addition to studying the variation in local sweating within and between subjects using the same locus of measurement, we also sought to determine the magnitude of potential differences in sweating between bilateral sites on the ventral forearms. Large differences in regional SRs among body sites have been reported (26, 27). If significant differences in local sweating exist between bilateral regions, it could affect the experimental design of investigations studying sweating, particularly those utilizing interventional treatments. However, if differences in SRs measured simultaneously between arms receiving the same sudomotor stimulus are no larger than day-to-day variation for the same arm, simultaneous bilateral measures could be made using one limb as a control. Two particular examples include the study of the latency period that extends between sweat gland stimulation and sweat emergence (4), which requires consideration of sweat gland priming (3, 25), and the study of the potential impact of topical skin protectants on

Address for reprint requests and other correspondence: R. W. Kenefick, US Army Research Institute of Environmental Medicine, Thermal and Mountain Medicine Division, Kansas St. Natick, MA 01760 (e-mail: robert.kenefick@us.army.mil).

sweating (11, 17). In fact, bilateral symmetry of the forearm sweating response has been assumed when using one forearm as the control while studying pharmacologically induced sweating in the other forearm (38).

Thus, the purpose of this study was to examine the components of biological variation for local steady-state forearm SR and sweating onset (SO) and their application for the future study of thermal physiology. Additionally, we sought to compare bilateral local forearm SR and SO simultaneously to determine the utility of comparing bilateral measures when it would be helpful to study design.

MATERIALS AND METHODS

Subjects. Ten healthy military volunteers (8 men and 2 women) participated in this study (age, 26 ± 9 yr; height, 176.4 ± 10.0 cm; weight, 79.9 ± 12.9 kg; body fat, 16.8 ± 8.6%; body surface area, 2.0 ± 0.2 m^2). Appropriate institutional review boards approved this study. Before participation, each volunteer attended briefings informing them of the purpose of the experiment and possible risks and completed a written informed consent document. Investigators adhered to policies for protection of human subjects as prescribed in Army Regulations 70-25 and US Army Medical Research and Materiel Command Regulation 70-25. The research was conducted in adherence with the provisions of 45 Code of Federal Regulations Part 46.

Experimental design and testing. The volunteers were partially heat acclimatized as the study was conducted in the summer months and the volunteers participated in military physical fitness programs. Volunteers completed a 5-day heat-acclimation protocol to minimize day-to-day variability in sweating within volunteers (i.e., changes in sweating due to acclimation/adaptation), in addition to improving exercise-heat tolerance and reducing the risk of heat exhaustion (33). Heat acclimation consisted of five consecutive days of 100-min treadmill walks (1.56 $m \cdot s^{-1}$, 4% grade; 1.6 l/min $\dot{V}O_2$ or 545 watts) in 45°C, 20 ± 2.0% RH, 1 $m \cdot s^{-1}$ wind speed. Volunteers wore the Army Improved Physical Fitness Uniform or equivalent (shorts and t-shirt; clo = 0.56, i_m/clo = 0.81). During heat acclimation and all experimental testing sessions, heart rate (HR) and rectal temperature (T_{re}) were measured every 10 min using a Polar HR monitor (model Polar a_3; Polar Electro, Woodbury, NY) and a telemetric temperature sensor (VitalSense Jonah ingestible capsule; Minimitter, Bend, OR) inserted 8–10 cm (length of gloved index finger) beyond the anal sphincter (18).

Following heat acclimation, volunteers performed three experimental trials separated by 3 to 5 days. Subjects continued their normal physical fitness program on the days between trials. Each experimental trial consisted of standing for 20 min, followed by walking for 30 min (1.6 $m \cdot s^{-1}$, 5.0% grade; 645 watts) on a treadmill windward to 1 $m \cdot s^{-1}$ wind speed, in a 40°C, 20% RH environment. This hot-dry environment was selected to maximize heat loss via evaporative sweating. In addition, the vapor pressure difference between moist skin and the ambient air was designed to be kept above the critical value (P_{crit}) for constant evaporation (2) to reduce skin wettedness and prevent sweat drippage from the skin. During each of the three trials, local measures of SR and SO were made on the same forearm. Sweating measures on both the right and left forearms were made during two trials. All trials were performed at the same time of day to control for circadian fluctuations in body temperature and other biological variables (37). Volunteers wore Army Combat Uniforms with sleeves rolled up to the elbow (forearms exposed; clo = 1.37, i_m/clo = 0.34) and running shoes during each of these trials, and no fluids were provided during the trials. The women who participated in the study were anovulatory; thus it was not necessary to control for menstrual cycle phase. Volunteers were given 2 liters of commercial sports drink to consume the evening before each experimental trial. One liter was consumed in the presence of an investigator in the evening and consumption of the second liter was verified on the morning of each trial. In addition, following each experiment, fluids lost were replenished (commercial sports drink) with fluid intake, such that subjects were rehydrated within 1% of initial body mass before leaving the laboratory.

Measurement description. Local steady-state SR and SO were measured from an automated enclosed ventilated 15.9 cm^2 capsule containing a microhumidity sensor (Honeywell HIH 4010) and a YSI 2.252 kΩ thermistor for temperature measures (13). This commonly used capsule technology (8, 22, 28, 34, 39) uses a polymer film with a dielectric constant that changes as a function of relative humidity. Pilot work in environmental conditions and work rates under which testing occurred were used to determine the capsule ventilation rate of 1.5 l/min via a rotameter (Aalborg, Orangeburg, New York) and the vapor pressure within the capsule. Room humidity was measured with a similar capsule hung near the volunteer. Before entering the environmental chamber, the midpoint of both the right and left ventral forearm (between the antecubital space and the wrist) was determined and cleaned with an alcohol pad, and a ~15.9-cm^2 circle was drawn with the midpoint as its center. Volunteers then entered the environmental chamber where the capsules were then affixed using Velcro straps placed around each forearm to the predrawn circle for the measurement of local forearm SR.

Local SRs were calculated (in $g \cdot cm^{-2} \cdot min^{-1}$) as: $SR = (\Delta H_2O)(AF)/(Rw \cdot A \cdot T)$ where ΔH_2O = water vapor pressure gradient (arm capsule vs. capsule in chamber, Torr), where AF is capsule air flow (l/min), Rw is gas constant for water vapor (3.464 $Torr \cdot l \cdot g \cdot K^{-1}$), A is area of capsule enclosing skin site (cm^2), and T is absolute room air temperature (K), as originally described (13). Values were then multiplied by 10^3 to give $mg \cdot cm^{-2} \cdot min^{-1}$. A 30-s sampling rate was selected, as this interval is well within the latency period that extends between sweat gland stimulation and sweat emergence (4). It also represents a fair tradeoff between sample frequency and sample noise (5). Local sweating measures were begun after 10-min of standing in the test environment to allow stable rest conditions. Steady-state SR was calculated as the average SR over the final 5 min of exercise. To compare local SR and SO responses over time between the right vs. left arm, the area under the curve (AUC) for the entire local sweating response (comprised of final 10 min of standing rest and the 30-min of walking) was calculated using the trapezium rule [$\Delta SR\ (t)\ dt$]. There is no consensus methodology in the literature for analysis of sweating sensitivity and interpretations among laboratories may be inconsistent (5). SR by time is commonly examined (1, 15, 19, 20, 23, 25, 26, 36, 37), and its relationship conforms to a mathematical expression defined by a plateau with a one-phase exponential association. Thus, to eliminate ambiguity in the determination of SO, SO time was determined objectively as the mathematical starting point for the exponential curve (24) using common curve analysis software.

Analytical variation. To determine the ventilated sweat capsule measurement system CV_a, measurements of SR were made in an environmentally controlled chamber equipped with a sweating guarded hot plate (Measurement Technology Northwest, Seattle, WA) on five separate occasions. The environmental chamber was set at 40°C, 20% rH and hot plate temperature was set at 36°C, closely approximating the testing environment and anticipated volunteer skin temperatures. A sweating membrane was prepared with deionized water and was secured to the hot plate, and a constant rate of deionized water was fed to the plate targeted to achieve a 1.5–2 $mg \cdot cm^{-2} \cdot min^{-1}$ SR similar to that anticipated during later experiments. This sweat rate was based on those observed during pilot work using the same environmental conditions as those during experimental testing. Prior to each measurement session, environmental conditions were stable, the hot plate primed, and steady-state sweating was achieved. Capsules were the same as were used in experimental testing and were ventilated at a rate of 1.5 l/min. SR measures were then made over five 4-min periods, and an average of the SRs was

calculated. The CV_a for sweat capsule measurement system performance was then calculated from the five independent measures using the naïve estimator (29) for the coefficient of variation [(SD/mean)*100].

Biological variation. The total within-subjects (CV_i) and between-subjects (CV_g) variation were computed by ANOVA according to published methods (10). The magnitude of dynamic change required to make a difference statistically significant, known as the reference change value (RCV), was calculated as [$RCV = 2^{1/2} \cdot Z \cdot (CV_a^2 + CV_i^2)^{1/2}$] (Z is the number of standard deviations appropriate to the desired probability), using a one-tailed test of significance in the direction of biological plausibility. Four probabilities were selected (0.80, 0.90, 0.95, and 0.99), which imply increasing strength against the null hypothesis (H_o). Although this is a strict misinterpretation of the *P* value complement (1 - P) (12), it communicates a more familiar and intuitive probability construct (6, 9). The starting value of 0.80 was considered the minimum for importance (6). For visual display a line relating probability (*Y*) with SR or SO (*X*) was best fit with the equation $Y = 1 - e^{-K \cdot X}$ (24). Against this background of probabilistic change, the magnitude of effects reported for SR and SO in response to various physiological perturbations (e.g., dehydration, age, and altitude exposure) can be interpreted. When calculating RCV, no consideration was made concerning the homogeneity of within-subjects variances (14), because no diagnostic or population extrapolations (6) were planned. The explanation for this rests on the educated assumption (15, 35) that a probabilistic nomogram for sweating, similar to what has been proposed for dehydration (6), would lose validity under circumstances that altered sweating appreciably from the conditions studied herein. The index of individuality was calculated as the ratio $(CV_a^2 + CV_i^2)^{1/2}/CV_g$ (9) to describe the relative proportions of variance within and between subjects.

Statistical analyses. Differences among repeated trials for SR and SO were examined using a one-way repeated-measures ANOVA and Tukey post hoc procedure when a significant *F* value was observed. Differences between local forearm SRs and onset times were investigated using paired *t*-tests, as were AUC comparisons. We considered meaningful any differences larger than the typical within-subjects SD. Although this affords a large effect size (≥1.0), it remains smaller than many differences reported in the literature for perturbations in sweating related to dehydration (23) or altitude exposure (20). An analysis selecting conventional α (0.05) and β (0.20) parameters showed that six subjects would provide sufficient power to detect the desired effect. Ten volunteers were tested to guard against the potential for missing data due to attrition.

RESULTS

All volunteers completed the 5-day heat-acclimation protocol and all experimental and control trials. Volunteers demonstrated classic physiological responses to repeated exercise-heat exposure, which included a significant ($P < 0.05$) decline in HR and T_{re} measures from *day 1* vs. *day 5* (147 ± 18 vs. 140 ± 15 beats/min, and 38.27 ± 0.25 vs. 37.81°C, respectively). In addition, there was also a significant ($P < 0.05$) increase in *day 1* vs. *day 5* SR (1.11 ± 0.25 vs. 1.24 ± 0.26 l/h) and a nonsignificant ($P > 0.05$) increase in walk time to exhaustion (87 ± 18 vs. 93 ± 12 min). Volunteers were considered euhydrated as the deviation in morning pre-experimental trial body mass was < 1.0%. Pretrial body mass measures were 80.3 ± 13.3 kg, 80.3 ± 13.0 kg, and 80.2 ± 13.6 kg ($P > 0.05$), indicating that volunteers were equally hydrated prior to each experimental trial. T_{re} and HR values were not different ($P > 0.05$) at rest or during exercise among the three trials. Following 20 min of standing in 40°C, the mean T_{re} and HR for all trials was 37.2 ± 0.16°C and 90 ± 9 beats/min. Resting local forearm SRs during the last 10 min of standing were ~0.5 $mg \cdot cm^{-2} \cdot min^{-1}$ in all trials ($P > 0.05$). At the completion of exercise, the mean T_{re} and HR for all trials were 37.97 ± 0.21°C and 148 ± 13 beats/min.

Analytical and biological components of variation. The CV_a for SR of the sweat capsule measurement system was 2.4% at the targeted 1.5–2 $mg \cdot cm^{-2} \cdot min^{-1}$ SR. As the mathematical precision for repeated measures for SO on the same data gives a difference of zero, the CV_a for SO was 0%. The CV_i for SR was 22.3% and for SO was 9.6%. The CV_g for SR was 56.4% and for SO was 41.0%. The ratio of variance within and between subjects, or the index of individuality was 0.40 for SR and 0.23 for SO (Table 1).

Local forearm SR and SO: repeated and bilateral measures. Figure 1 depicts the mean values and absolute range for SR and SO for each of the 10 volunteers over the 3 days of testing. The local forearm mean SR values were not different ($P = 0.19$) over 3 days of testing (2.14 ± 0.72 vs. 2.02 ± 0.79 vs. 2.31 ± 0.72 $mg \cdot cm^{-2} \cdot min^{-1}$). Local forearm SO values were also not different ($P = 0.82$) over the 3 days of testing (10.47 ± 2.54 vs. 10.04 ± 2.97 vs. 9.87 ± 3.44 min).

Figure 2 depicts the left and right arm differences in local SR and SO plotted against the line of identity and in relation to the combined analytical and within-subject biological variation $(CV_a^2 + CV_i^2)^{1/2}$. Bilateral SR (2.14 ± 0.72 vs. 2.21 ± 0.70 $mg \cdot cm^{-2} \cdot min^{-1}$, $P = 0.56$) and SO (10.47 ± 2.54 vs. 10.83 ± 2.48 min, $P = 0.09$) were similar and between-day differences (SR = 0.07 ± 0.4 $mg \cdot cm^{-2} \cdot min^{-1}$; SO = 0.35 ± 0.62 min) were ≤ 1 SD of day-to-day differences for a single forearm (Table 1).

Figure 3*A* represents SR measures over time for the left and right arms for the last 10-min of standing rest and 30-min of walking exercise. The histogram in Figure 3*B* depicts the bilateral comparison of the AUC values for SR for left and right arms over the entire observation period. There were no differences ($P = 0.29$) between SR AUC for left (58.2 ± 21.4 $mg \cdot cm^{-2}$) vs. right (55.6 ± 22.1 $mg \cdot cm^{-2}$) forearm measures between the two trials.

Figure 4 depicts the change (*x*-axis) in local forearm SR and SO plotted as a function of the statistical probability (*y*-axis)

Table 1. *Components of analytical and biological variation*

	CV_a		CV_i		CV_g		$(CV_a^2 + CV_i^2)^{1/2}/CV_g$
Quantity	%	Units	%	Units	%	Units	Ratio
Sweat rate, $mg \cdot cm^2 \cdot min^{-1}$	2.4	0.04	22.3	0.44	56.4	1.22	0.40
Sweat onset, min	0*	0.00*	9.60	0.98	41.0	4.16	0.23**

*Mathematical precision for repeat measures on the same data gives difference of zero; **Ratio for Sweat onset reduces to CV_i/CV_g when CV_a is negligible. Note: $CV_a < 0.5(CV_i)$ increases $CV_i \leq 3\%$; e.g., CV_a 11% and CV_i 22% results in $(11^2 + 22^2)^{1/2}$ = total measurement variation of 25%. CV_a, analytical imprecision; CV_i, within-; and CV_g, between-subjects' coefficient of variation.

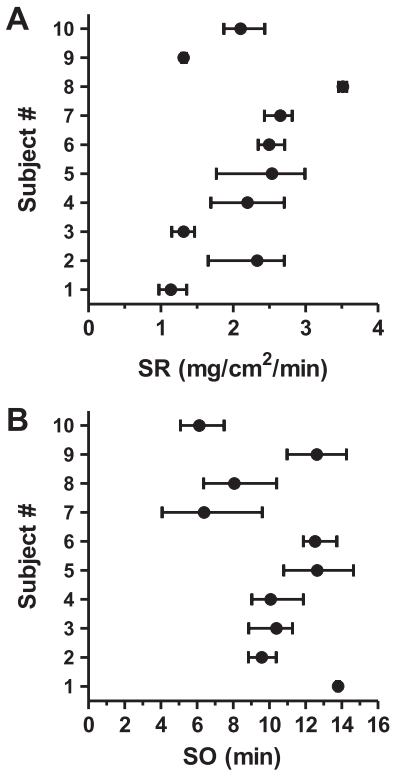

Fig. 1. Mean values (dot) and absolute range (bars) for local forearm sweating rate (SR: *A*) and sweating onset (SO; *B*) for 10 volunteers measured 3 times over 2 wk (30 measures). The differences between mean values represent the variation between subjects, while the individual range around each mean represents the variation within subjects.

that the change is significant using one-tailed Z values in the direction of biological plausibility. Thus, they are depicted as positive changes in sweating but could apply equally to anticipated negative changes in sweating. A line with the equation $Y = 1 - e^{-K \cdot X}$ best fit the data for visual display (24). The gray areas represent the CV_i. The dashed projection in Fig. 4*B* extends beyond the RCV 0.99 probability range to accommodate larger changes reported in the literature. The letter "a" represents the changes reported in SR and SO for dehydration (% body mass loss) (23), heat acclimation (1, 16, 36), age (1), and menstrual cycle (19). The letter "b" represents the changes reported in SR and SO for high altitude exposure (>4,500 m) (20) and marked dehydration (5% body mass loss) (23). It is important to be aware that observed changes that fall within the CV_i (gray area) are likely not meaningful. Also, note that the largest change for SR (0.54 $mg \cdot cm^{-2} \cdot min^{-1}$) (19), relative to the random day-to-day fluctuation (CV_i), gives an effect size (ES) of 1.0, but most studies show smaller effects (ES < 0.25). For SO, the smallest ES is > 4.0. According to the equation: ES·(sample size)$^{1/2}$ (30), 10 times as many subjects are required to have the same statistical confidence in the measured outcome when using SR instead of SO.

DISCUSSION

This investigation was the first to describe the biological variability for local SR measures and to determine the CV_a of a ventilated sweat capsule measurement system used to measure SR and SO under carefully controlled environmental conditions. Local SR and SO were selected because they are common thermoregulatory effector response measurement, and knowledge of its biological variability is important for designing experiments and interpreting results. In addition, this is the first investigation to experimentally compare bilateral local forearm SR and SO. The investigation was carefully designed to control preanalytical factors such as circadian rhythm, hydration state, exercise intensity, and acclimation status. Based on the results of our experiments we conclude the following: *1*) sweat capsules contribute negligibly to sweat measurement variation, *2*) bilateral measures of SR and SO appear to be interchangeable, *3*) changes in SO afford a more favorable signal-to-noise ratio compared with SR when studying factors that potentially affect sweating. These results have important implications for estimating sample size, effect magnitude, and choice of research designs including use of interchangeable bilateral measures when appropriate.

While modern laboratory methods are commonly associated with small analytical variation (9), to our knowledge the CV_a of modern ventilated sweat capsule sweat measurement system used in experimental research (8, 22, 28, 34, 39) has never been determined. This is the first investigation to ascertain the CV_a of ventilated sweat capsules measurement system employing unique methodology via an environmentally controlled chamber and sweating guarded hot plate in environmental conditions similar to those used during testing. In the present investigation, the CV_a of the sweat capsule measurement system was 2.4% for SR and ~0% for SO. As previously stated, knowledge of CV_a is important because it represents bias introduced

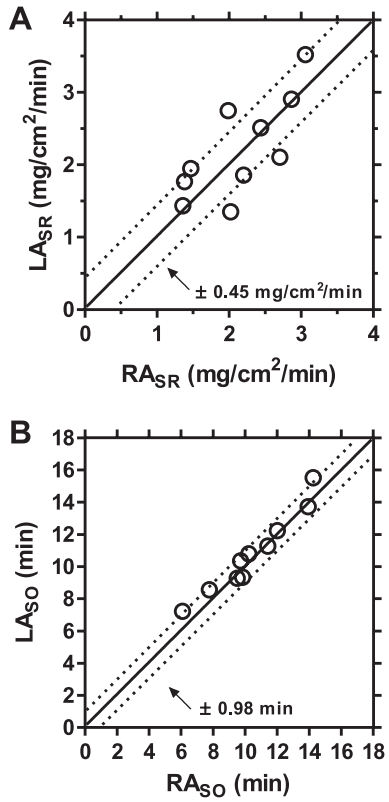

Fig. 2. Bilateral (right vs. left arm) differences in local forearm SR (*A*) and SO (*B*) plotted against the line of identity and relative to the $(CV_a^2+CV_i^2)^{1/2}$ (dotted lines) for each measure. LA, left arm; RA, right arm.

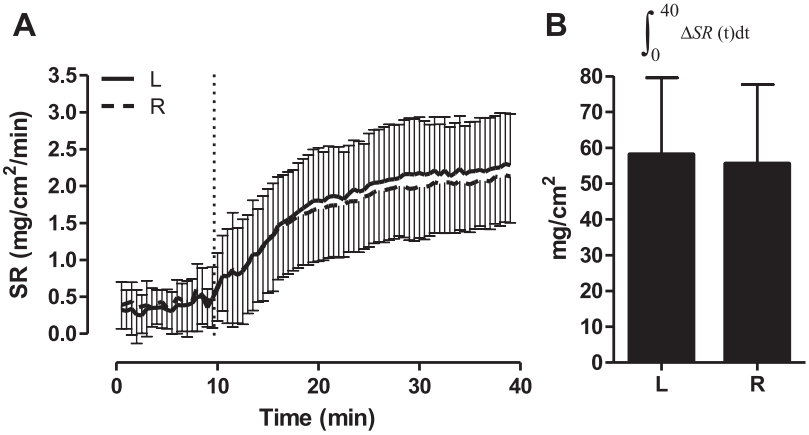

Fig. 3. Local forearm SR measures over time for the LA and RA for the last 10 min of standing rest and 30-min of walking exercise are depicted in *A* where the dotted line indicates the start of exercise. The histogram in *B* depicts the comparison of the area under the curve values for SR over time for LA and RA during the last 10 min of standing rest and 30 min of walking exercise.

by the instrument itself each time a measurement is made (9). It has also been suggested that the CV_a of an ideal sweat capsule measurement system should be $< 50\%$ of the CV_i to be acceptable and $< 25\%$ of the CV_i to be optimal (9). As can be seen in Table 1, the CV_a for both SR and SO were $< 25\%$ of the CV_i for both measures adding a maximal variability of 3% to an individual measure (9) (see Table 1 for sample calculation). These findings demonstrate that the ventilated sweat capsule measurement system used in local measurements of SR and SO contribute negligibly to total sweat measurement variation.

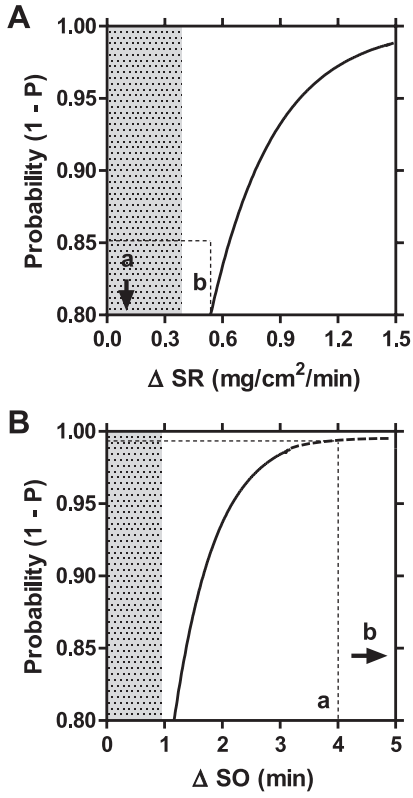

Fig. 4. Change (*x*-axis) in local forearm SR (*A*) and SO (*B*) plotted as a function of the statistical probability (*y*-axis) that the change is significant. The gray areas represent the CV_i. The CV_i for SR was larger than most of the perturbations reported by many of the studies in the literature for SR (1, 16, 19, 23, 36). The dashed projection in *B* extends beyond the reference change value (RCV) 0.99 probability range to accommodate larger changes in the literature. The letter "a" represents the changes reported in the literature for SR (*A*) and SO (*B*) for dehydration (23), heat acclimation (1, 16, 36); age (1), and menstrual cycle (19). The letter "b" represents the reported changes in SR (*A*) and SO (*B*) due to high altitude exposure (>4,500 m) (20) and severe dehydration (5% body mass loss) (23). Observed changes that fall within the CV_i (gray area) are likely not meaningful. CV_i, within subjects' coefficient of variation.

This is also the first investigation to examine bilateral differences for SR and SO. In the present investigation, SR and SO of the left and right forearms were equivalent and bilateral differences were within 1 SD of day-to-day differences of a single forearm (Fig. 2), indicating that bilateral SR and SO measures are interchangeable (Fig. 3). The concept that large bilateral differences in SR and SO may exist is not unfounded. Anatomical differences in sweat gland density (31) and differences in regional SRs among body sites have been reported (26, 27). Despite these reported differences, we observed no differences in SR or SO between arms, over repeated days when using the same anatomical locus. This observation may have an important application to the design of investigations studying sweating either driven by pharmacological intervention or via thermoregulation. In particular, studies requiring a comparable control for an interventional treatment (e.g., drugs, topical emollients) could utilize the contralateral limb/body segment, thereby reducing difficulties associated with repeated day testing in controlling preanalytical factors (hydration state, circadian rhythm, sex hormones, sleep) and save in cost and time.

The CV_i observed in our study is only the second to be reported for local SR (15) and the first to be reported for SO time. The values we observed for SR (23.3%) exclude noise from the CV_a but are still greater than three times larger than the ~6% reported for the forearm by Hayden et al. (15). There are many distinctions between our study and that of Hayden et al., including differences in sudomotor drive and absolute SR due to discrepancies in heat load, as well as differences in the technology used to measure SR. Hayden et al. (15) also calculated the CV using the naive estimator (29), which slightly underestimates the true CV (7, 29). Knowledge of the CV_i for both SR and SO is important as the reproducibility of any thermoregulatory effector response is a key to understanding the potential effects of other variables on that parameter (3). Greater variation in CV_i and CV_g as a result of sex could be a concern with the inclusion of two women in the study cohort. However, when the two women were removed from

analysis, the change in CV_i and CV_g was not appreciably different and was no greater than when two random men were removed from the analysis.

The potential utility of using biological variation to understand thermoregulatory sweating has far-reaching implications for research study design and methodology (15), statistical analysis, and sample size considerations. The CV_i for SR (22.3%) and SO (9.6%) were relatively low. As can be seen in Fig. 4*A*, the CV_i for SR was larger than most of the perturbations reported by many of the studies in the literature for SR (1, 16, 19, 23, 36). Furthermore, the probability that these changes in SR were significant was generally low (< 0.80) with one exception (20), which indicates a small effect magnitude (or type I error when $P < 0.05$) as the changes fall within the CV_i. In contrast, as depicted in Fig. 4*B*, the perturbations reported in the literature for SO were well outside the CV_i and the probability that these changes were significant were > 0.95 (or type II error when $P > 0.05$).

What can be taken from this analysis is that SO is far more sensitive to laboratory perturbations in thermoregulatory sweating than SR. By way of example, analysis of the mean data presented by Montain et al. (23) shows that SO is delayed by almost 1 min for each 1% loss of body mass by sweating (dehydration), whereas the same perturbation altered SR by only 0.08 $mg \cdot cm^{-2} \cdot min^{-1}$. SO is also commonly examined and can be determined using a simple, objective method (24) free of bias inherent in other methods (5). For example, there is no consensus methodology in the literature for analysis of sweating sensitivity and the choice of which phase of the sweating response to analyze (early vs. late) contributes to greater measurement variation and inconsistencies among laboratories performing these analyses (5). In addition, the core temperature threshold for sweating has already been shown to be highly reproducible (0.1°C) within subjects (3). In contrast, SR is a simple, unambiguous measure commonly made using precision sweat capsules (5, 13) and SO (1, 16, 17, 19, 23, 36) is also commonly used and can be objectively determined.

Finally, the index of individuality, or the ratio $(CV_a^2 + CV_i^2)^{1/2}/CV_g$, is typically < 1.0 since variability between subjects is greater than variability within subjects for most biological measures (9). This was also the case in the present study, where the variability in both SR and SO was substantially greater between than within subjects (Table 1). The importance of this ratio relates to the design of experiments and sample size estimates for between-, rather than within-group research designs, such as when making sex or age-related comparisons in sweating. It is important to note that the SR and SO measures reported in this investigation are specific to the environmental conditions utilized during testing and the exercise workload employed, both of which can greatly impact P_{crit} (2). The impact of fitness, acclimation state, age, and other phenotypic characteristics (e.g., body surface area) of the volunteers, not to mention factors such as sweat gland ultrastructure, sweat gland density, and neurotransmitter release/responsiveness on CV_g would logically be minimized by group stratification (9). The effects on CV_i are presumably much smaller since these factors would not change from day to day for the same person. Although the CV_a among laboratories might differ due to use of varied flow rates or differences in capsule size, the small CV_a observed herein suggests labs using common systems that contain the same sensors to measure humidity and temperature should achieve comparable imprecision. However, every laboratory should know (and report) the CVa for the setup they use, similar to how CV_a is commonly reported for biochemistry assays.

Perspective and Significance

On the basis of the findings of this investigation, we conclude that given the low CV_a, ventilated sweat capsules measurement systems introduce negligible sweat measurement variation. Also, bilateral measures of SR and SO appear to be interchangeable, which has important applications/implications to study design, particularly those involving interventional treatments where a comparable control is required. Finally, given the smaller variation in SO and the large impact (effect magnitude) of common perturbations on SO (1, 16, 19, 23, 36), it appears to be a very sensitive measure for studying thermoregulatory effector responses. In addition, SO would require fewer volunteers than SR when estimating study sample size (30). Although the probability curves and associated change values for SR and SO (Fig. 4) are specific to the conditions tested herein, the principal contributions of this and recently related works (5) will assist thermal physiologists in the quantitative and qualitative study of thermoregulation.

ACKNOWLEDGMENTS

The authors thank the volunteers who donated their time and effort to participate in this study. The authors also thank Kurt Sollanek for technical assistance and Dr. Nisha Charkoudian for editorial contributions.

All experiments were carried out in accordance to state and federal guidelines. The views, opinions, and/or findings in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision unless so designated by other official designation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.W.K., S.N.C., L.D.E., and M.N.S. conception and design of research; R.W.K., S.N.C., L.D.E., and B.R.E. performed experiments; R.W.K., S.N.C., L.D.E., and B.R.E. analyzed data; R.W.K., S.N.C., L.D.E., and M.N.S. interpreted results of experiments; R.W.K., S.N.C., and B.R.E. prepared figures; R.W.K., S.N.C., and B.R.E. drafted manuscript; R.W.K., S.N.C., L.D.E., B.R.E., and M.N.S. edited and revised manuscript; R.W.K., S.N.C., L.D.E., B.R.E., and M.N.S. approved final version of manuscript.

REFERENCES

1. **Armstrong CG, Kenney WL.** Effects of age and acclimation on responses to passive heat exposure. *J Appl Physiol* 75: 2162–2167, 1993.
2. **Berglund LG, Gonzalez RR.** Evaporation of sweat from sedentary man in humid environments. *J Appl Physiol* 42: 767–772, 1977.
3. **Brengelmann GL, Savage MV, Avery DH.** Reproducibility of core temperature threshold for sweating onset in humans. *J Appl Physiol* 77: 1671–1677, 1994.
4. **Bullard RW.** Studies on human sweat gland duct filling and skin hydration. *J Physiol (Paris)* 63: 218–221, 1971.
5. **Cheuvront SN, Bearden SE, Kenefick RW, Ely BR, DeGroot DW, Sawka MN, Montain SJ.** A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. *J Appl Physiol* 107: 69–75, 2009.
6. **Cheuvront SN, Ely BR, Kenefick RW, Sawka MN.** Biological variation and diagnostic accuracy of dehydration assessment markers. *Am J Clin Nutr* 92: 565–573, 2010.
7. **Chow SC, Tse SK.** Outlier detection in bioavailability/bioequivalence studies. *Stat Med* 9: 549–558, 1990.

8. **Crandall CG, Musick J, Hatch JP, Kellogg DL Jr, Johnson JM.** Cutaneous vascular and sudomotor responses to isometric exercise in humans. *J Appl Physiol* 79: 1946–1950, 1995.
9. **Fraser CG.** *Biological Variation: From Principles to Practice.* Washington, DC: American Association of Clincal Chemistry, 2001.
10. **Fraser CG, Harris EK.** Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 27: 409–437, 1989.
11. **Gabaree CLV, Mair BS, Kolka MA, Stephenson LA.** Effects of topical skin protectant on heat exchange in humans. *Aviat Space Environ Med* 68: 1019–1024, 1997.
12. **Goodman SN.** Toward evidence-based medical statistics. 1: The *P* value fallacy. *Ann Intern Med* 130: 995–1004, 1999.
13. **Graichen H, Rascati R, Gonzalez RR.** Automatic dew-point temperature sensor. *J Appl Physiol* 52: 1658–1660, 1982.
14. **Harris EK.** Distinguishing physiologic variation from analytic variation. *J Chronic Dis* 23: 469–480, 1970.
15. **Hayden G, Milne HC, Patterson MJ, Nimmo MA.** The reproducibility of closed-pouch sweat collection and thermoregulatory responses to exercise-heat stress. *Eur J Appl Physiol* 91: 748–751, 2004.
16. **Henane R, Flandoris R, Charbonnier JP.** Increase in sweating sensitivity by endurance conditioning in man. *J Appl Physiol* 43: 822–828, 1977.
17. **Kenefick RW, Cheuvront SN, Ely BR, Palombo LJ, Sawka MN.** DEET insect repellent: effects on thermoregulatory sweating and physiological strain. *Eur J Appl Physiol*. In press.
18. **Kenefick RW, Ely BR, Cheuvront SN, Palombo LJ, Goodman DA, Sawka MN.** Prior heat stress: effect on subsequent 15-min time trial performance in the heat. *Med Sci Sports Exerc* 41: 1311–1316, 2009.
19. **Kolka MA, Stephenson LA.** Control of sweating during the human menstrual cycle. *Eur J Appl Physiol* 58: 890–895, 1989.
20. **Kolka MA, Stephenson LA, Rock PB, Gonzalez RR.** Local sweating and cutaneous blood flow during exercise in hypobaric environments. *J Appl Physiol* 62: 2224–2229, 1987.
21. **Lenth R.** Some practical guidelines for effective sample size determination. *Am Stat* 55: 187–193, 2001.
22. **Lorenzo S, Minson CT.** Heat acclimation improves cutaneous vascular function and sweating in trained cyclists. *J Appl Physiol* 109: 1736–1743, 2010.
23. **Montain SJ, Latzka WA, Sawka MN.** Control of thermoregulatory sweating is altered by hydration level and exercise intensity. *J Appl Physiol* 79: 1434–1439, 1995.
24. **Motulsky H, Christopoulos A.** *Fitting Models to Biological Data Using Linear and Nonliner Regression: A Practical Guide to Curve Fitting.* New York: Oxford University Press, 2003.
25. **Nadel ER, Bullard RW, Stolwijk JAJ.** Importance of skin temperature in the regulation of sweating. *J Appl Physiol* 31: 80–87, 1971.
26. **Nadel ER, Mitchell JW, Saltin B, Stolwijk JAJ.** Peripheral modifications to the central drive for sweating. *J Appl Physiol* 31: 828–833, 1971.
27. **Patterson MJ, Galloway SD, Nimmo MA.** Variations in regional sweat composition in normal human males. *Exp Physiol* 85: 869–875, 2000.
28. **Patterson MJ, Stocks JM, Taylor NA.** Humid heat acclimation does not elicit a preferential sweat redistribution toward the limbs. *Am J Physiol Regul Integr Comp Physiol* 286: R512–R518, 2004.
29. **Quan H, Shih WJ.** Assessing reproducibility by the within-subject coefficient of variation with random effects models. *Biometrics* 52: 1195–1203, 1996.
30. **Sackett DL.** Why randomized controlled trials fail but needn't: 2. Failure to employ physiological statistics, or the only formula a clinician-trialist is ever likely to need (or understand!). *CMAJ* 165: 1226–1237, 2001.
31. **Sato K, Kang WH, Saga K, Sato KT.** Biology of sweat glands and their disorders. I. Normal sweat gland function. *J Am Acad Dermatol* 20: 537–563, 1989.
32. **Sawka MN, Gonzalez RR, Pandolf KB.** Effects of sleep deprivation on thermoregulation during exercise. *Am J Physiol Regul Integr Comp Physiol* 246: R72–R77, 1984.
33. **Sawka MN, Wenger CB, Pandolf KB.** Thermoregulatory responses to acute exercise-heat stress and heat acclimation. In: *Handbook of Physiology, Environmental Physiology*. New York: Oxford University Press, 1996, sect. 4, p. 157–185.
34. **Shibasaki M, Inoue Y, Kondo N, Aoki K, Hirata K.** Relationship between skin blood flow and sweating rate in prepubertal boys and young men. *Acta Physiol Scand* 167: 105–110, 1999.
35. **Shibasaki M, Wilson TE, Crandall CG.** Neural control and mechanisms of eccrine sweating during heat stress and exercise. *J Appl Physiol* 100: 1692–1701, 2006.
36. **Shido O, Sugimoto N, Tanabe M, Sakurada S.** Core temperature and sweating onset in humans acclimated to heat given at a fixed daily time. *Am J Physiol Regul Integr Comp Physiol* 276: R1095–R1101, 1999.
37. **Stephenson LA, Wenger CB, O'Donovan BH, Nadel ER.** Circadian rhythm in sweating and cutaneous blood flow. *Am J Physiol Regul Integr Comp Physiol* 246: R321–R324, 1984.
38. **Vimieiro-Gomes AC, Magalhaes FC, Amorim FT, Machado-Moreira CA, Rosa MS, Lima NR, Rodrigues LO.** Comparison of sweat rate during graded exercise and the local rate induced by pilocarpine. *Braz J Med Biol Res* 38: 1133–1139, 2005.
39. **Wilson TE, Cui J, Crandall CG.** Mean body temperature does not modulate eccrine sweat rate during upright tilt. *J Appl Physiol* 98: 1207–1212, 2005.